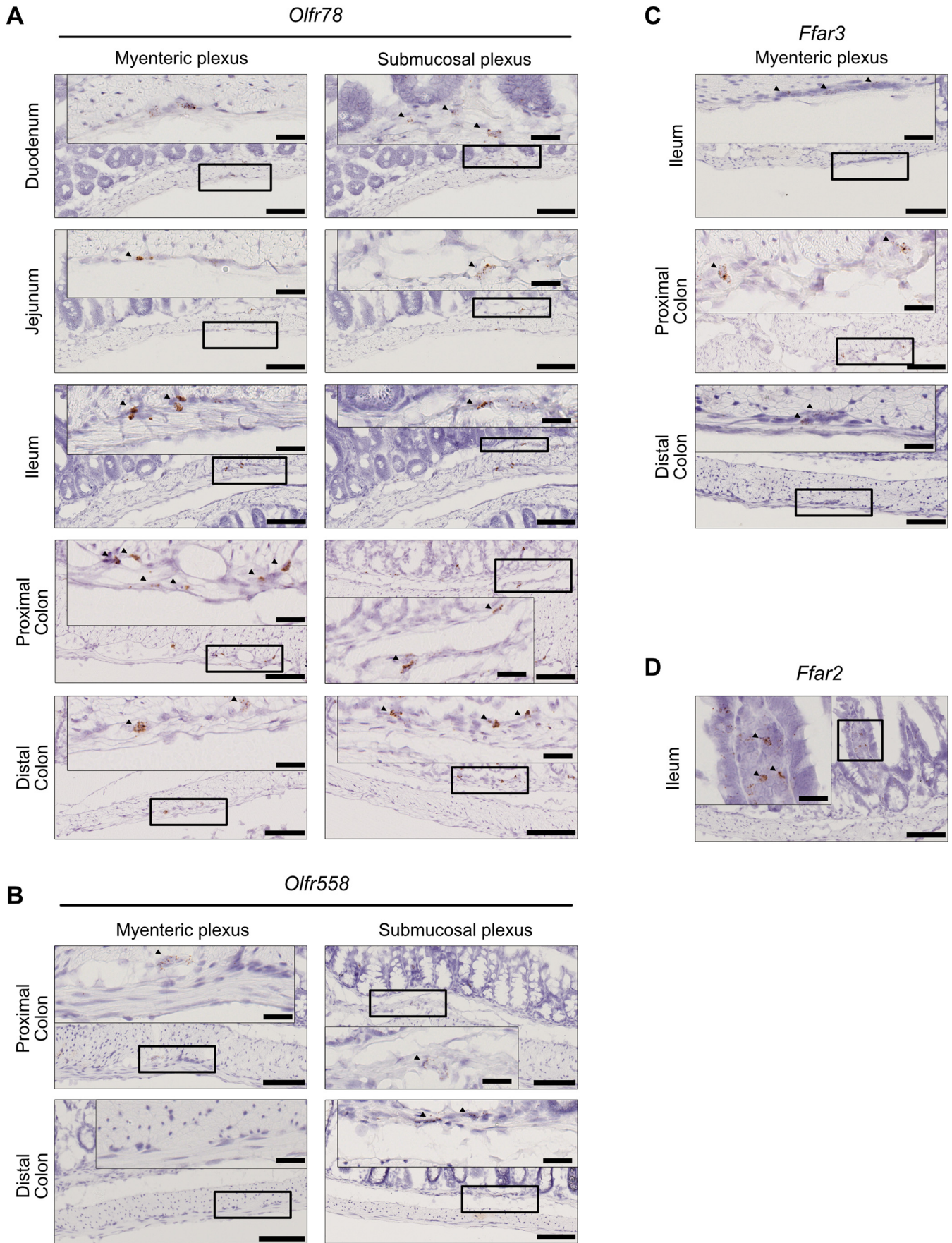
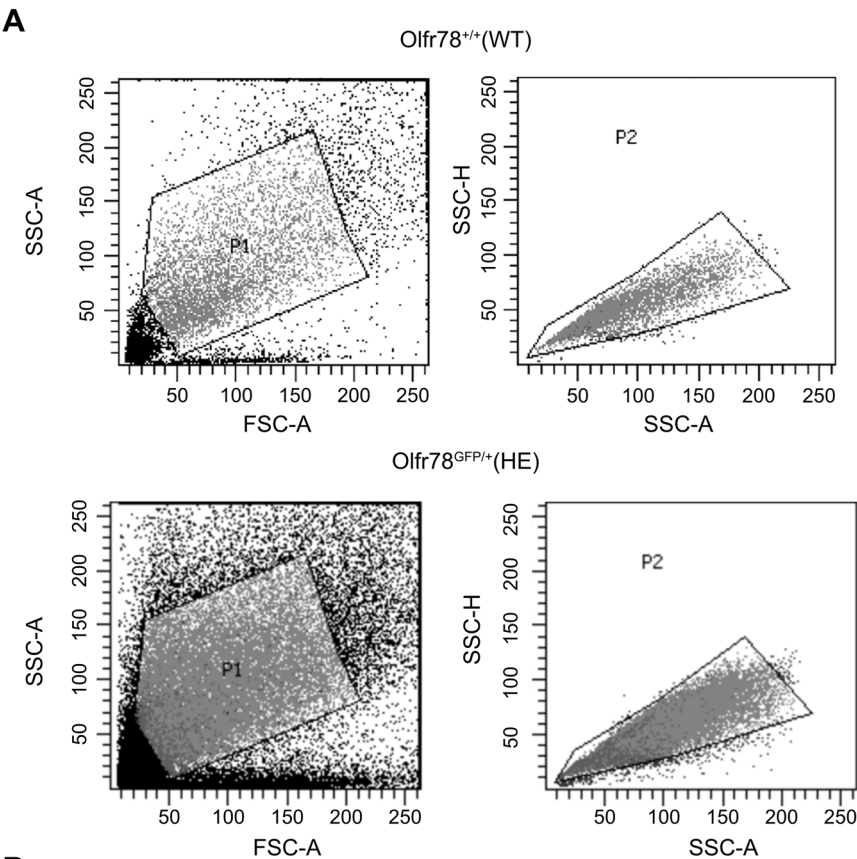


Expanded View Figures

Figure EV1. SCFA receptors exhibit unique expression profiles along the small intestine and colon.

(A) Representative RNAscope pictures of mesenchymal expression of Olfr78 in the gut. (B) Representative RNAscope pictures of mesenchymal expression of Olfr558 in the colon. (C) Representative RNAscope pictures of Ffar3 expression in myenteric plexuses in the gut. (D) Representative RNAscope pictures of mesenchymal expression of Ffar2 in the ileum. Data information: Scale bars: 100 μm (low views) or 25 μm (insets). Arrowheads identify isolated SCFA-expressing cells.

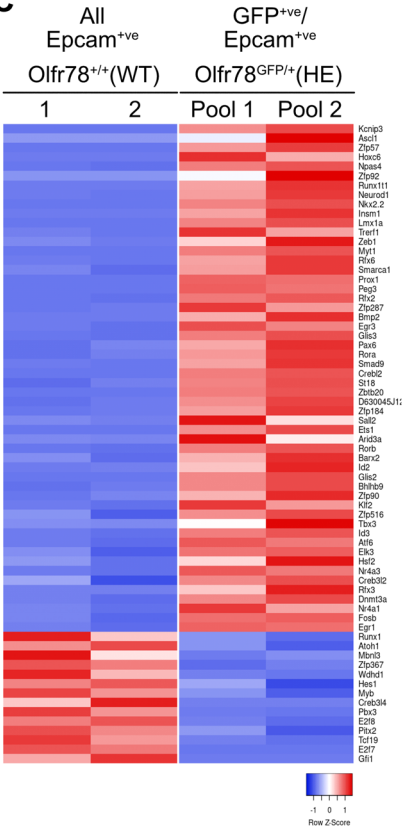




B

Up		
Gene	Log2FC	FDR
Amigo2	9.15848307	1.11E-114
Slc18a1	6.79241159	1.67E-103
Celf3	7.61408531	2.95E-100
Trph1	6.61902534	9.38E-98
Cpe	6.44741623	2.23E-94
Chga	6.36548722	1.77E-91
Man1c1	6.74444704	1.58E-87
Map1b	6.49923649	2.73E-87
Neurod1	6.84702226	7.93E-85
Klf1a	6.01803633	7.50E-83
Chab	6.20893923	1.07E-82
1810006J02		
Rik	7.14903542	2.40E-82
Wif1	6.76009617	3.47E-80
Ptprn	6.37906213	2.98E-78
Gnap1	6.34003023	1.25E-77
Cerkl	6.96501199	5.09E-77
Runx11	6.95662951	5.95E-75
Rimbp2	5.56144189	3.18E-73
Pcsk1n	7.34781804	2.14E-72
Slc38a11	5.71212377	1.96E-70
Down		
Lpo	-4.08743095	2.35E-51
mt-Atp8	-3.96886994	4.88E-43
mt-Atp6	-3.51520681	8.00E-35
mt-Co2	-3.27504437	1.36E-28
Nov	-2.91734831	1.18E-26
Klk1	-2.84714055	2.51E-26
Gm28439	-3.05479056	4.89E-24
Spink4	-2.80673265	3.79E-20
Tg	-11.6632431	3.27E-19
Acpp	-5.45734642	7.55E-19
Sult1c2	-3.42806109	2.31E-17
Gm10222	-2.80082698	4.17E-17
Hells	-2.87316541	1.38E-16
Hmqb2	-2.40677088	2.46E-16
mt-Co3	-2.55896806	3.03E-16
Krt14	-4.37611909	3.99E-16
Mntx1	-2.29087238	4.37E-16
Edn1	-3.28661953	6.58E-16
Svalf1	-3.3470063	1.72E-15
Gm1123	-2.59013523	5.57E-15

C



◀ **Figure EV2. Olfr78 is expressed in different subtypes of enteroendocrine cells in the colon.**

(A) FACS strategy for initial population selection and doublets exclusion. (B) List of the 20 most up or downregulated genes in Epcam⁺/⁺GFP⁺ cells as compared to all Epcam⁺ cells, ranked by FDR. (C) List of significantly up and downregulated transcription factors in Epcam⁺/⁺GFP⁺ cells as compared to all Epcam⁺ cells.

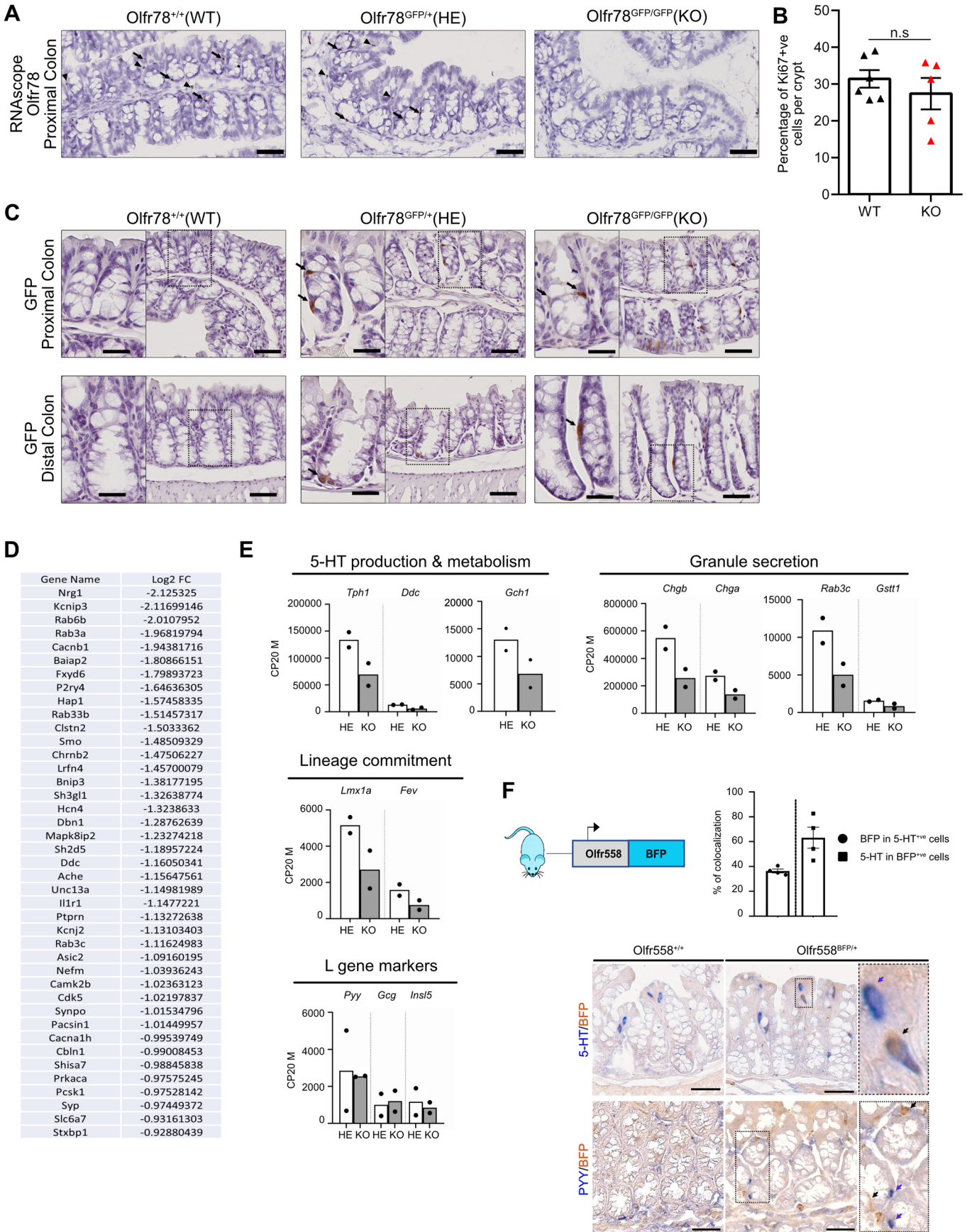
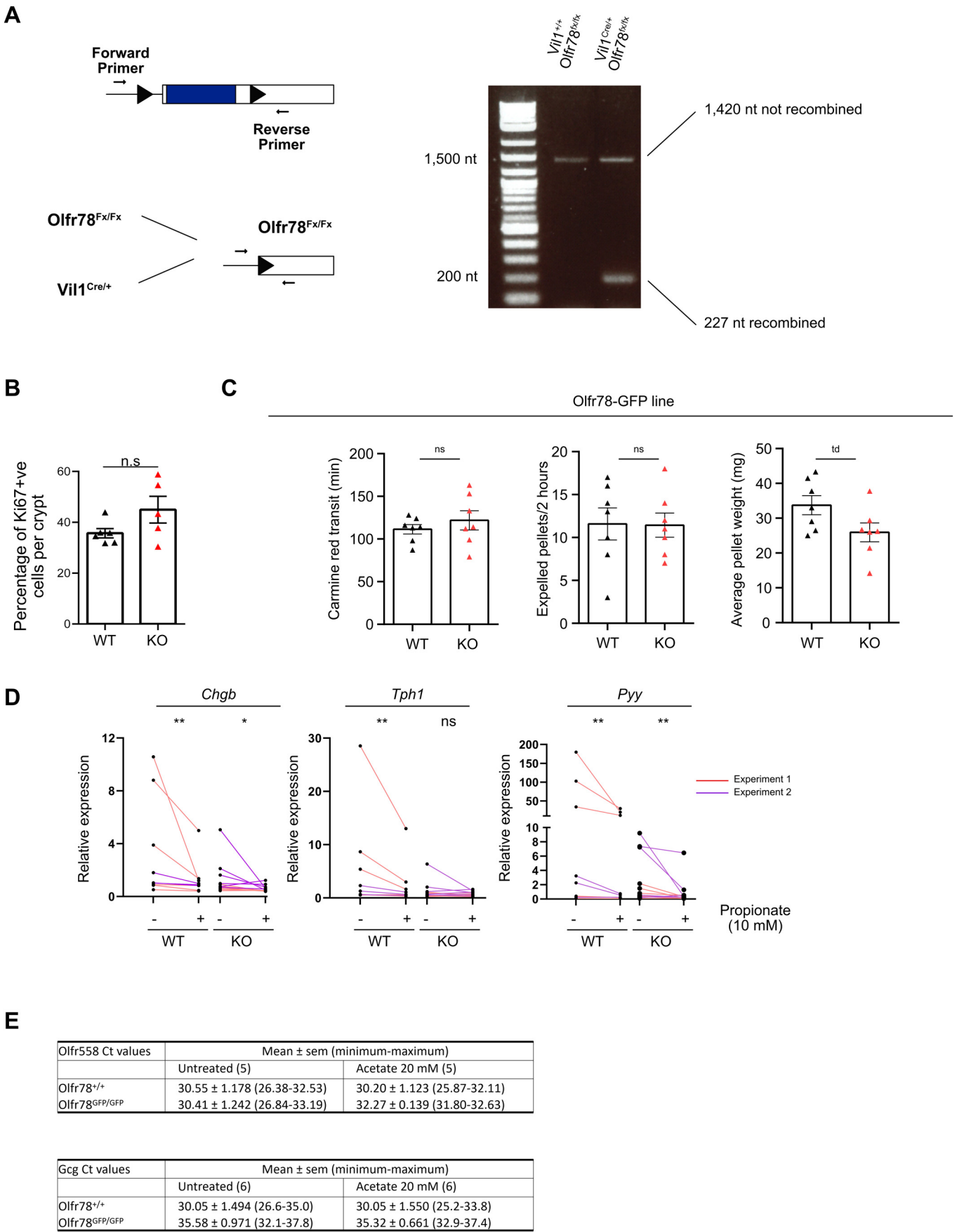


Figure EV3. Loss of Olfr78 impairs terminal differentiation into enterochromaffin cells.

(A) Olfr78 expression in proximal colon of WT, HE and Olfr78-GFP KO mice analyzed by RNAscope. Arrows and arrowheads identify epithelial and mesenchymal Olfr78-expressing cells, respectively. (B) Quantification of cell proliferation (Ki67⁺ cells) in proximal colon crypts of Olfr78-GFP WT and KO mice. Each symbol indicates the value for a given mouse ($n = 6$ WT, 5 KO). (C) GFP expression in proximal colon of WT, HE and Olfr78-GFP KO mice analyzed by Immunohistochemistry. (D) List of downregulated genes in Olfr78-GFP KO Epcam⁺/GFP⁺ cells related to GSEA pre- or post-synapse gene lists, ranked by Log₂(Fold Change). (E) Histograms showing the expression of target genes in the bulk RNAseq from Fig. 3D. CP20M: counts per 20 million mapped reads. (F) Upper panel: Quantification of colocalization between 5-HT or PYY and BFP in the newly generated Olfr558-BFP mouse line (BFP cassette replacing the Olfr558 coding region). Each symbol indicates the value of a given mouse ($n = 4$). Lower panel: Representative pictures of double immunohistochemistry showing 5-HT/BFP and PYY/BFP co-stainings in proximal colon of Olfr558-BFP WT or HE mice. Data information: Scale bars: 50 μ m (A, C and F) or 25 μ m (A and C, inset). Data are represented as mean $\pm$ SEM.; unpaired t-test, n.s = not significant.



◀ **Figure EV4. Terminal differentiation into serotonin-producing cells is regulated by epithelial Olfr78 expression.**

(A) Left: PCR strategy for loxp sites recombination verification in $Vil1^{Cre/+}$ - $Olfr78^{fx/fx}$. Right: Gel electrophoresis showing WT and recombinant bands in $Vil1^{Cre/+}$ - $Olfr78^{fx/fx}$. (B) Quantification of Ki67⁺ cells in proximal colon crypts of $Olfr78$ -GFP WT and KO mice. Each symbol indicates the value for a given mouse. (C) Histograms showing the transit time of carmine red (left), the number of fecal pellets expelled during 2 h (middle) and the average wet stools weight (right) in $Olfr78$ -GFP WT or KO mice. Each symbol indicates the value for a given mouse ($n = 7$ WT and 7 KO). (D) Graphs showing the relative expression levels of EEC markers analyzed by qRT-PCR on colon organoids after 48 h of treatment with propionate at 10 mM. Data are reported as the relative expression levels in treated and control conditions in $Olfr78$ -GFP WT and KO organoids. Each symbol indicates the individual value of a given organoid line in each experiment ($n = 3$ -6 WT and 6 KO). Colored lines identify paired samples in 2 independent experiments. (E) Raw cycle threshold values obtained from qPCR experiments performed on $Olfr78$ -GFP WT or KO organoids (mean $\pm$ SEM). Data information: Data are represented as mean $\pm$ SEM.; (B, C) unpaired t-test n.s = not significant; td: tendency ($p = 0.0673$), (D) Wilcoxon matched-pairs signed rank test, n.s = not significant, * $P < 0.05$, ** $P < 0.01$.

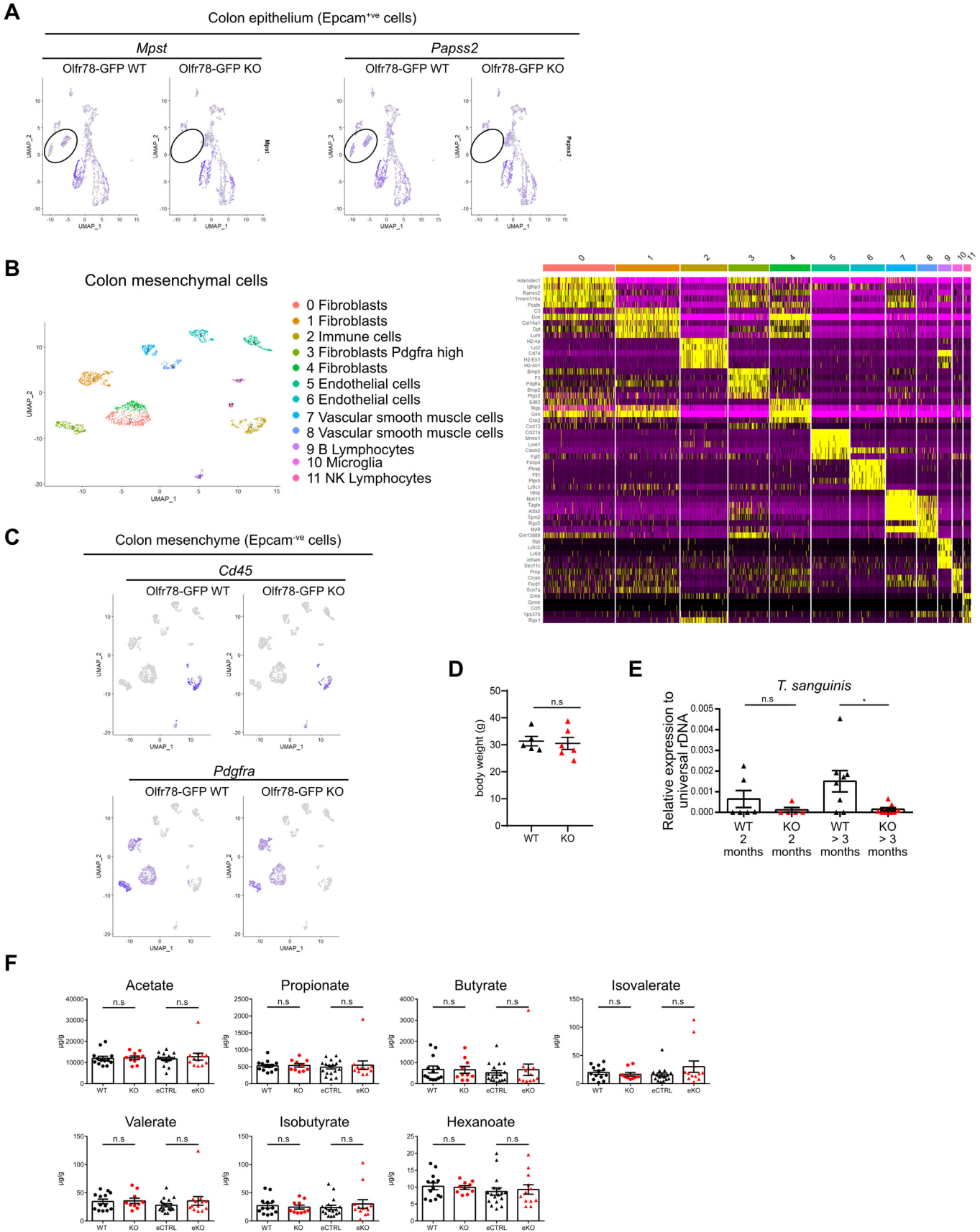


Figure EV5. Loss of Olfr78 expression alters colon homeostasis.

(A) Umap of Olfr78-GFP WT and KO colon epithelial cells showing *Mpst* or *Papss2* expression. Circles identify enriched clusters differentially present in WT and KO mice. (B) Left panel: Merged UMAP of colon mesenchymal cells from Olfr78-GFP WT and Olfr78-GFP KO mice. Right panel: Heatmap showing the top 5 markers of each cluster of the UMAP. (C) Umap of Olfr78-GFP WT and KO colon mesenchymal cells showing *Cd45* or *Pdgfra* expression. (D) Weight of adult Olfr78-GFP mice. Each symbol indicates the value for a given mouse ($n = 5$ WT and 6 KO). (E) Analysis of *Turicibacter sanguinis* prevalence by qPCR in the fecal microbiota of Olfr78-GFP WT or KO mice at different ages. Each symbol indicates the value for a given mouse ($n = 6-8$ WT and 5-9 KO). (F) Quantification of fecal SCFA concentrations. Each symbol indicates the value for a given mouse (Olfr78-GFP line: $n = 13$ WT and 10 KO; VilCre/Olfr78-Fx line: $n = 17$ controls and 12 eKO). Data information: Data are represented as mean $\pm$ SEM.; (D, E, F) Mann-Whitney tests, (E), n.s = not significant; * $P < 0.05$.